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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

This progress report details our four months research effort to develop and show feasibility of biosensors for detection of toxic compounds. Progress to date in the following areas is reported: 1) selection of pSD721 lux-gene E. coli transformant for detection of carcinogens, 2) optimization of photoluminescent response by this clone, 3) stabilization and immobilization of E. coli biosensors by lyophilization, and 4) fabrication and testing of a portable photodiode light detection system. Keywords: Bioluminescence (AW)

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PRINCIPAL INVESTIGATOR: Dr. Reinhardt A. Rosson

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RESEARCH OBJECTIVE: To demonstrate the feasibility of bioluminescent testing for detection of toxic compounds and to develop an inexpensive photodiode based light detection system for measurement of low level bioluminescence.

WORK IN PROGRESS: Since our last technical report progress has been made on several fronts including: optimization of the bioluminescent response to ethidium bromide, development of a light detection system, evaluation of several potential immobilization methodologies, and initial investigation of the *lux/mer* gene fusion.

1. Detection of carcinogens using cloned *lux* genes in *E. coli*.

We have transformed pSD721, using electroporation, into a variety of *E. coli* hosts with different genetic backgrounds. Three clones (*E. coli*DH5α[pSD721], *E. coli*LE392[pSD721], and *E. coli*WB373[pSD721]) were selected for further study because of their relatively low endogenous background luminescence as well as their rapid response to low levels of the carcinogen, ethidium bromide (EtBr). For simplicity, we will refer to strain designation when referring to these clones, e.g. DH5α for *E. coli*DH5α[pSD721].

To date all experiments have been carried out in a rich growth medium, Luria Broth (LB) at 30°C. LB supports rapid growth of these clones. Biosensor response to EtBr was measured by taking 1 ml of freshly grown and diluted cultures in LB at 30°C for a variety of cell densities (measured by optical density at 600 nm (OD_{600})), and placing these cultures in glass vials so that the bacterial suspension formed a thin film across the bottom of the vial. The EtBr solution was added and the kinetics of the response over a period of approximately 60 min, without agitation, was monitored using a photomultiplier; this configuration most accurately models the anticipated biosensor applications. In order to optimize biosensor response, we determined the effect of clone suspension density, as well as the effect on diluent of expression of luminescence.

As shown in Figure 1, all three clones at OD_{600} = 0.5 show a significant response to 20 ppm of EtBr, however with the DH5α showing a detectable response even at the 2 ppm level. It should be noted that the optimization of this response to EtBr has just been initialized and further improvements are anticipated. Figures 2 through 4 show the response of fully induced DH5α clones grown to an OD_{600} = 1 (late exponential growth) and then diluted in conditioned medium (cell free LB which pSD721 clones were grown to an OD_{600} = 1). At all three cell densities background luminescence remains at a low level. The addition of EtBr at time zero induces a rapid increase in luminescence, which is easily measurable in 10 minutes or less at a cell concentration OD_{600} = 0.5. The cell population doubles approximately every 60 minutes (with agitation) so the luminescent response measured was affected significantly by the presence of EtBr. The DH5α clone showed an easily measurable significant response to 2 ppm of EtBr and much higher levels of sensitivity should be available with further optimization. Oxygen is a substrate for luminescence. Potential biosensors may be in static systems with low O_2 availability, therefore lower cell density is a prime consideration for some applications. At lower cell densities, initial response to EtBr was measurable after only 20 minutes and was very strong for the DH5α clone.

Results from these initial studies have led to the selection of DH5α for immobilization studies, to be performed during the remainder of the Phase I contract period.

2. Immobilization and light detection

The DH5α clone has been lyophilized at a variety of cell densities in conditioned LB. Studies are in progress to determine the viability of the response after storage under a variety of storage conditions (ie. temperature and light variation). Response of the lyophilized cells to EtBr, after rehydration, is also being determined under similar conditions.

Several light detection systems have been tested with varying degrees of success, but several economical photodiode based systems have proven capable of measuring the bioluminescence response. Design of the field detection system is dependent on the oxygen and temperature requirements for optimized response. With the completion of the EtBr lyophilization experiments, a final design for the initial field system can be completed.

3. Mercury biosensor development

We have obtained plasmids and bacteriophage containing cloned bioluminescence (*lux*) and mercury (*mer*) genes, respectively and verified the presence and orientation of these genes. A 6.6 Kbp DNA fragment from the *lux* operon of *Xenorhabdis luminescens* on the plasmid pCGLS11 (*lux* operon in plasmid pUC19) will be used for *mer/lux* fusion. Two M13 page containing cloned genes of the broad spectrum mercury resistance operon originally the plasmid pDU1358 (from a *Serratia* sp.) have been obtained. One of these contains

the regulatory gene, *mer*R, the operator promotor (OP) region, and the beginning of the first transport genes *mer*T. The second phage contains *mer*R, OP, both transport genes *mer*T and *mer*P and part of the reductase gene *mer*A.

We have purified *lux* plasmid and *mer* phage DNA and verified that these DNAs can be cut with restriction enzymes. We are now ready to do the genetic engineering to fuse these two genes. The resulting *lux/mer* fusion can be transformed into an appropriate *E. coli* host to generate a mercury biosensor. Our strategy is to excise the *mer* genes from the M13 phage with Pvu I and Sal I. pCGLS11 will be linearized with Sma I and Sal I, and the *mer* DNA will be ligated into the Sal/Sma site of pCGLS11 forming a new plasmid with the *lux* and *mer* genes fused together. These will be transformed into *E. coli* DH5α to form a biosensor.

4. Projected research plan

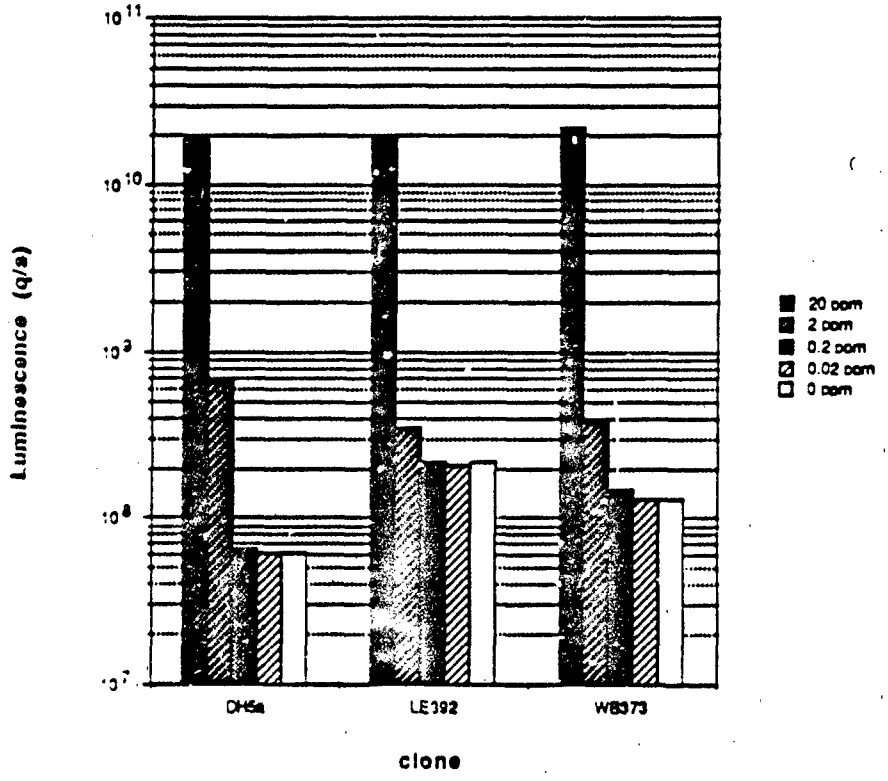
The success of these initial tests has shown that photoluminescent clones are a viable detector for trace concentration of specific chemicals. The remainder of this initial study will focus on the utilization of lyophilized DH5α clones in several types of conditioned media (ie. complex and minimal). In addition, tests with fully induced cells in a nonconditioned minimal media will also be conducted.

The lyophilized DH5α clones will be used in studies of potential interferences using a variety of inhibitors as well as a study of response to other intercalating substances. A variety of common chemicals and inhibitors likely to be present in environmental samples will be tested on lyophilized DH5α clones to determine possible problems of interference with the response to EtBr.

Once the mercury biosensor is constructed, we will complete initial testing of its response and sensitivity to concentration of mercury. This work is particularly significant as it illustrates the potential applicability of the photoluminescent techniques to the production of biosensors for a vast number of specific chemicals.

Figure 1

Luminous Response of pSD721 Clones After 70 Minute Exposure to Ethidium Bromide at Various Concentrations (Culture OD600 = 0.5)



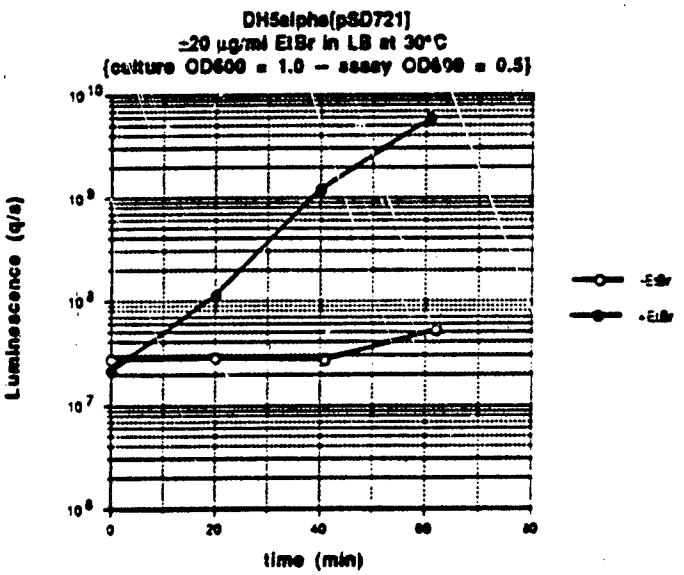


Figure 3

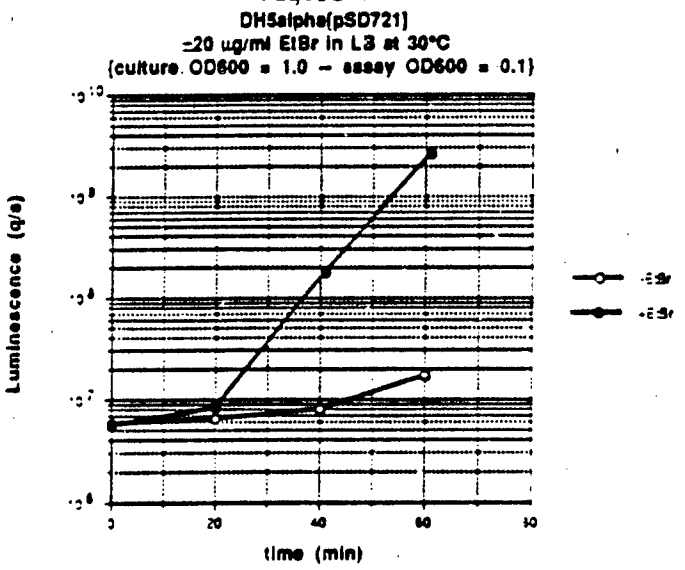
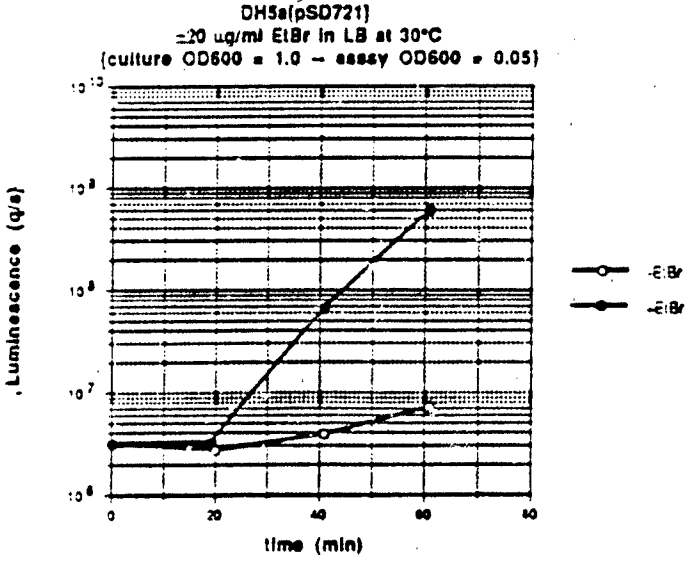


Figure 4



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